(300B) and 1-(2,2-Bis-phosphono-ethyl)-3-butyl-pyridinium (476A) are 4.68 μM and 6.48 μM respectively. Isothermal Calorimetric studies with these inhibitors revealed that the binding is entropically driven. X-ray crystallographic structures of the LmFPPS in complex with 300B, Isopentenyl Pyrophosphate (IPP), and 3 divalent cations and the other in complex with 476A, IPP and 3 divalent cations were determined. Comparison of these structures with those of the human enzyme complexed with zolendronate reveals significant differences in residues at the bottom of the pocket, such as Glu97 and Leu129, that could be used to tailor specificity of the bisphosphonates to the parasitic enzyme.

1195-Pos Board B105
Computational Enzyme Design: Refining Artificial Enzymes and Exploring Paths of Directed Evolution
Maria P. Frushicheva, Arieh Warshel.
A fundamental challenge in biotechnology and in biochemistry is the ability to design effective enzymes. In fact, it would be one of the most convincing indications of a full understanding of the origin of enzyme catalysis. Despite a gradual progress on this front, most of the advances have been made by placing the reacting fragments in the proper places rather than by optimizing the environment preorganization, which is the key factor in enzyme catalysis (Chem. Rev. 2006, 106, 3210-3235). Improving the preorganization and assessing the effectiveness of different design options requires the ability to calculate the actual catalytic effect. Our studies are based on using the empirical valence bond (EVB) as the main screening tool (for e.g., Biochemistry 2009, 48, 3046-3056). At present this approach appears to provide the most reliable way for quantifying catalytic effects. Previously, we explored the challenges of the rational enzyme design in artificial Kemp eliminases with glutamate as a base (Frushicheva et al., PNAS, Sept 09 2010). It has been shown that due to the small change in substrate charges upon reaching the transition state makes it hard to exploit the active site polarity. This appears to be the case even with the ability to quantify the effect of different mutations. Thus we study the catalytic effect of the Kemp elimination reaction with different catalytic bases, such as histidine and lysine. This allowed us to exploit change in basicity as well the larger dipole of the transition charge distribution. We also investigate the effect of mutations that convert the highly catalytic phosphotriestrase to a lactonase. Overall we are able to illustrate the effectiveness of the EVB as a powerful approach for a quantitative screening in rational enzyme design.

1196-Pos Board B106
Mechanical Tuning of a Catalytic Cystein PKA in Thioredoxin
Pallav Kosuri, **Bo Chen**, Arne Holmgren, Julio M. Fernández.
Protein function is often modulated by the protonation state of functional groups. Thioredoxin is a universally conserved disulfide reductase with wide substrate specificity, and its catalytic activity is dependent on the deprotonation of Cys32. This cysteine exhibits a pK_a that is markedly lower than free cysteine, ensuring catalytic activity at physiological p*H*. Various electrostatic effects within the active site have been suggested to cause this pK_a depression. However, a consensus on the actual pK_a value is still lacking, as reported values have differed depending on the method used. We show here that the pK_a of Cys32 in human thioredoxin can be modulated by its substrate. We used an atomic force microscope to detect single disulfide cleavage events in immunoglobulin substrate proteins. Integrating the data from many such events for a range of p*H* conditions enabled precise measurement of the rate of nucleophilic attack by thioredoxin, which is linearly dependent on nucleophile concentration. Fits to the Henderson-Hasselbalch equation allowed for sensitive titration of the Cys32 pK_a. Interestingly, when altering the mechanical strain on the substrate we observed a concomitant change in the enzyme pK_a. We could in this manner continuously tune the Cys32 pK_a between 6.5 and 7.5. These results suggest that the conformation of the substrate influences the enzyme pK_a. Electrostatic interactions in the enzyme-substrate binding interface could be responsible for this modulation. The substrate dependence of the Cys32 pK_a could explain why previous reports have failed to reach a consensus on its value. Our findings may indicate a general phenomenon, which brings into question the relevance of measuring enzyme pK_a's in the absence of physiological substrates.

1197-Pos Board B107
Heptosyltransferase I Lipid a Structure Activity Relationships (SAR)
Daniel J. Czyzyk, Erika A. Taylor.
The increasing incidence of antibiotic resistant bacterial infections has necessitated the search for novel targets and mechanisms for therapeutic intervention. Formation of a biofilm enables survival of bacteria in hostile environments, including in the presence of antimicrobials, thereby prompting research toward the disruption of bacterial biofilms. Lipopolysaccharide (LPS), a major constituent of the outer membrane of gram-negative bacteria, plays a key role in the formation and stability of biofilms. Therefore, disrupting the biosynthesis of the LPS is an attractive mechanism for development of anti-biofilm agents. One such LPS biosynthetic enzyme is heptosyltransferase I (Hep I), which is responsible for the transfer of the first L-*glycero*-D-*manno*-heptose to a 3-deoxy-α-D-*manno*-oct-2-ulopyranosonic acid (KDO) of the growing Lipid A portion of LPS. Previous attempts to co-crystallize Hep I with both substrates have been unsuccessful, hindering the ability to effectively visualize the binding interactions important for specificity. Substrate analogues for *E. coli* Hep I Lipid A are being used to identify which portions are necessary and sufficient for binding. Determination of the SAR of Hep I for these analogues may lead to the discovery of a Lipid A analogue that is able to be co-crystallized with Hep I. Developing an understanding of *E.coli* Hep I binding interactions with lipid A will enhance efforts to develop a Hep I inhibitor and possibly the discovery of a new anti-biofilm agent.

1198-Pos Board B108
Mechanistic Study of Dihydropteridine Reductase (DHPR) Using Raman Spectroscopy
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Dihdyropteridine reductase (DHPR) catalyzes reduction of the unstable quinonoid dihydropteridine to the active tetrahydropteridine form through the oxidation of NADH. DHPR deficiency or any blockage in biosynthesis of tetrahydropteridine results in development of phenylketonuria (PKU), a progressive neurological illness which does not respond to dietary treatment. It has been speculated that DHPR or metabolites associated with it may have antioxidative properties. In another study, DHPR has been shown to have NADH-ferric reductase activity. This activity is postulated to have an important role in dietary iron uptake. There is an emerging role, though mechanistically unclear at this point, for BH4 in maintaining nitric oxide synthase (NOS) activity. NOS produces the signaling agent nitric oxide (•NO) from L-arginine. In the light of these emerging roles there is a growing need to completely understand how DHPR works.
The mechanism of catalysis in DHPR is not fully understood. Previous studies have suggested the involvement of the thiol group of a cysteine residue in DHPR. DFT computational modeling of Raman spectra of the unlabeled and isolabeled inhibitor of DHPR provides valuable information for assigning Raman marker bands which are involved in enzyme- inhibitor interactions. Using Raman spectroscopic techniques, the effect of the inhibitors/substrate and/or cofactor on the protonation state of the thiol group is investigated. Raman spectroscopy is particularly effective because of a unique peak at ca. 2500 cm-1 due to the S-H stretch and another one at ca. 900 cm-1 due to the C-S bond. Peak shifts and modulation in intensities are monitored and analyzed in terms of its mechanistic implications.

1199-Pos Board B109
Structural and Functional Studies of a New Isoform of Indoleamine 2, 3-dioxygenase
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Indoleamine 2, 3-dioxygenase-1 (IDO1) is a heme-containing enzyme that catalyzes the first and rate-limiting step of the kynurenine (KYN) pathway, the major catabolic route of L-tryptophan (L-Trp). Expression of IDO1 is induced by immunological stimuli (e.g. interferon gamma), causing T-cell inhibition. Therefore, IDO1 has been implicated in various physiological and patho-physiological processes, including viral infections and cancer. In cancer cells, IDO1 is expressed to help tumors evade immune surveillance, resulting in uncontrolled tumor growth and cancer progression. The IDO1 inhibitor, 1-methyl-tryptophan (1MT) has been known to inhibit mouse IDO1 and, together with chemotherapeutic agents, to reduce tumor size. However, additional tests revealed that the L-isomer of 1-MT has more potent inhibitory efficiency in vitro with purified IDO1, while the D-isomer is more potent in vivo. The discrepancy of these results was partially answered by the recent discovery of a new isoform of IDO1, named IDO2, which have been shown to be preferentially inhibited by D-1MT in vivo. Although, D-1MT is in phase I clinical trials, no structural or molecular studies of purified human IDO2 has been reported so far. Hence, there exists a critical need for the understanding of the structural and functional properties of IDO2, in order to clarify the physiological functions of IDO2 to provide guidelines for the development of new and potent drugs for cancer therapy. To obtain a better insight into the structure-function of IDO2 we have cloned, expressed and purified recombinant human IDO2. Our spectroscopic and kinetic data provide a glimpse of the structural and functional properties of IDO2 for the first time, and suggest that the action mechanism of IDO2 is distinctive from IDO1.